Marked-Up Version of Claims

1. A method of targeted delivery of mammalian stem cells of myeloid origin into a nervous system of a mammal by administering a therapeutically effective amount of mammalian stem cells of myeloid origin into said nervous system of said mammal, whereby

said mammalian stem cells of myeloid origin migrate from an injection site to a preferred site in said nervous system of said mammal, and

said mammalian stem cells of myeloid origin engraft into said nervous system of said mammal at said preferred site.

2. The method of Claim 1, wherein said mammalian stem cells of myeloid origin are isolated from at least one of the group of bone marrow, mobilized peripheral blood, umbilical cord blood, or fetal liver tissue from a mammal.

3. The method of Claim 1, wherein administration of said therapeutically effective amount of mammalian stem cells is at least one of the group of intrathecal, intraventricular, intracisternal, intraparenchymal into the brain or spinal cord, or systemic.

4. The method of Claim 1, wherein administration of said mammalian stem cells of myeloid origin is a combination of at least two of the group of intrathecal, intraventricular, intracisternal, intraparenchymal into the brain or spinal cord, or systemic.

5. The method of Claim 1, wherein said mammalian stem cells of myeloid origin maintain the pluripotential capacity to differentiate into neuronal and glial cells.

6. The method of Claim 1, wherein said mammalian stem cells are transiently or stably genetically engineered by at least one viral vector or non-viral transfection.

7. The method of Claim 1, wherein said mammalian stem cells of myeloid origin deliver viral vectors, other transducing agents, or biological pumps of peptides directly into said nervous system of said mammal.

8. The method of Claim 1, wherein said mammalian stem cells of myeloid origin comprises delivery of cells expressing CD34.

9. The method of Claim 1, wherein said mammalian stem cells of myeloid origin comprises delivery of cells negative for CD34.

10. A method of modifying neuronal growth [treating disorders, diseases, or trauma of a nervous system] of a mammal by administering a therapeutically effective amount of mammalian stem cells of myeloid origin into a [said] nervous system of said mammal, whereby

said mammalian stem cells of myeloid origin migrate from an injection site to a preferred site in said nervous system of said mammal,

said mammalian stem cells of myeloid origin engraft into said nervous system of said mammal at said preferred site,

said engrafted mammalian stem cells of myeloid origin differentiate into neuronal and glial cells, and

said neuronal and glial cells replace damaged nervous system tissue.

11. The method of Claim 10, wherein said mammalian stem cells of myeloid origin are isolated from at least one of the group of bone marrow, mobilized peripheral blood, umbilical cord blood, or fetal liver tissue from a mammal.

12. The method of Claim 10, wherein administration of said therapeutically effective amount of mammalian stem cells is at least one of the group of intrathecal, intraventricular, intracisternal, intraparenchymal into the brain or spinal cord, or systemic.

13. The method of Claim 10, wherein administration of said therapeutically effective amount of mammalian stem cells is a combination of at least two of the group of intrathecal, intraventricular, intracisternal, intraparenchymal into the brain or spinal cord, or systemic.

14. The method of Claim 10, wherein said mammalian stem cells are transiently or stably genetically engineered by at least one viral vector or non-viral transfection.

15. The method of Claim 10, wherein said mammalian stem cells of myeloid origin deliver viral vectors, other transducing agents, or biological pumps of peptides directly into said nervous system of said mammal.

16. The method of Claim 10, wherein said mammalian stem cells of myeloid origin comprises delivery of cells expressing CD34.

17. The method of Claim 10, wherein said mammalian stem cells of myeloid origin comprises delivery of cells negative for CD34.

18. A method of modifying neuronal growth [treating disorders, diseases, or trauma of a nervous system] of a mammal by administering a therapeutically effective amount of mammalian stem cells of myeloid origin into a [said] nervous system of said mammal, wherein said mammalian stem cells are transiently or stably genetically engineered by at least one viral vector or by non-viral transfection, whereby

said mammalian stem cells of myeloid origin migrate from an injection site to a preferred site in said nervous system of said mammal,

said mammalian stem cells of myeloid origin engraft into said nervous system of said mammal at said preferred site,

said engrafted mammalian stem cells of myeloid origin differentiate into neuronal and glial cells, and

said neuronal and glial cells replace damaged nervous system tissue.

19. A method of modifying neuronal growth [treating disorders, diseases, or trauma of a nervous system] of a mammal by administering a therapeutically effective amount of mammalian stem cells of myeloid origin into a [said] nervous system of said mammal, wherein said stem cells of myeloid origin deliver viral vectors, other transducing agents, or biological pumps of peptides directly into said nervous system of said mammal, whereby

said mammalian stem cells of myeloid origin migrate from an injection site to a preferred site in said nervous system of said mammal,

said mammalian stem cells of myeloid origin engraft into said nervous system of said

mammal at said preferred site,

said engrafted mammalian stem cells of myeloid origin differentiate into neuronal and glial cells, and

said neuronal and glial cells replace damaged nervous system tissue,

said mammalian stem cells of myeloid origin migrate from an injection site to a preferred site in said nervous system of said mammal,

said mammalian stem cells of myeloid origin engraft into said nervous system of said mammal at said preferred site,

said engrafted mammalian stem cells of myeloid origin differentiate into neuronal and glial cells, and

said neuronal and glial cells replace damaged nervous system tissue.

REMARKS

An amendment to the Final Office Action is submitted in which the Examiner's rejections have been considered. Claims 1-19 are pending in the application. Claims 10, 18, and 19 have been amended. Support for these amendments can be found on page 22, lines 13-14; page 22, line 25; page 23, lines 12-15; and page 23, lines 21-27. No new matter has been added.

Rejections under 35 U.S.C. § 112, 1st paragraph

Claims 1-19 have been rejected under 35 U.S.C. § 112, first paragraph. In particular, the Office Action states that:

> [t]he specification, while being enabling for methods drawn to mouse and rat models, does not reasonably provide enablement for methods drawn to the treatment of human subjects...

The Office Action further also asserts that:

> Applicant has not taught in the specification how one of skill in the art may overcome the unpredictability and lack of sophistication in stem cell therapy and gene therapy, such that one of skill in the art may practice the invention in all mammals, including humans. Applicant has merely provided examples in *Parkinsonian rats and mice without data or evidence supporting a correlation between these models and other mammals*, including humans. Therefore, Applicant has failed to fully enable the scope of the claimed invention. (Emphasis added.)

Applicants respectfully disagree with the rejection. However, to expedite prosecution of the application, Applicants have amended claims 10, 18, and 19 to remove the term "treating" and instead to recite "modifying neuronal growth." The specification describes that such modification results from cell migration into multiple target regions leading to differentiation into developmentally and regionally appropriate cells, such as neuronal and glial cells (See page 23, line 21-27). The results show that this modification occurs at particular desired regions, i.e. those that are damaged, and demonstrate that "cells show a natural ability to migrate away from the injection site, travelling preferentially to lesioned areas in all lesion models (i.e., lysolecithin, NMDA, 6-OHDA)." (See page 23, lines 29-31).

With regard to the issue of correlation between animal models and other mammals, Applicants describe the use of robust and art recognized models for Parkinson's Disease (PD). These PD animal models can be created, for example, by apomorphine, lysolecithin, NMDA, or 6-OHDA (See page 23, line 31 of the specification). At the time the invention was filed, these models were routinely used in the art as evidenced by the following representative abstracts and references:

1. Kolasiewicz *et al.* (1983) *Exp Neurol.* 81(1):195-209.
2. Carman *et al.* (1991) *Brain Res.* 553(2):275-283.
3. Strömberg *et al.* (1995) *Brain Res Bull.* 38(3):221-233.
4. Mandel *et al.* (2000) *Exp Neurol.* 161:212-219.

These references show that at the time the application was filed that the skilled artisan appreciated the existence of a correlation between the behavioral effects, "which are generally accepted as laboratory models of Parkinson's diseases..." (*See* Kolasiewicz *et al., Supra*). It is routinely accepted that these animal models of the disease are used as the initial stage for *in vivo* testing because they efficiently mimic the physiology and etiology of the disease. Applicant's specification uses a well established animal model of PD and describes in detail how to isolate and admit a therapeutically effective amount of cells to produce an ameliorative effect, as shown in the rotational studies of Figure 1. There is no reason to believe that Applicants' results in these art recognized models would not be predictive of a similar effect in other mammals. Moreover, the genomes of rodents and mammals are redundant such that studies done in rodents can be directly extrapolated to mammals; hence, studies in well-characterized animal models of disease are commonly used as the direct precursor to clinical trials. Rat and mouse models of CNS diseases have predicted primate and human data in fetal transplant studies as well as in CNS diseases (Freed, C. R. *PNAS* 99(4):1755-1757 (2002)). In his commentary on the Björklund *et al.* 2002 article, Freed points out that fetal dopamine cell transplantation is currently used as a "proven strategy for treatment of patients with advanced Parkinson's disease" and that "[a]ll of

the principles developed in the rat have been validated in human subjects" (See Freed, *Supra*, page 1755, column 3).

In addition, at the time of the invention, methods of cell replacement had been successfully used in both animal and human studies of various diseased states, including disorders associated with the nervous system. Specifically, clinical trials, utilizing cell replacement therapies for disorders of the central nervous system, such as Parkinson's disease (PD), Huntington's disease, epilepsy and stroke, were successfully being conducted (See, for example, Björklund *et al. Nature Neuroscience* 3 (6):537-544 (2000)). Results of cell replacement studies on Parkinson's disease and Huntington's disease confirm that the "results of human patient trials are generally consistent with findings in experimental animals" (Björklund *et al. Nature Neuroscience* 3 (6): 537-544 (2000); See page 538, column 3). In addition, Björklund *et al.* point out that "there are well-characterized rodent and primate models of PD, and although these have a different etiology from the human disease, they nevertheless mimic its cardinal features, and they have repeatedly proved to have good predictive value with respect to effects of therapeutic intervention on symptoms in PD patients" (See page 537, column 3).

Applicants have demonstrated successful transplantation of myeloid stem cells of human origin in well-characterized animal models. Moreover, Applicants have shown successful long-term transplantation of these human myeloid stem cells as well as improved behavior corresponding to the treatment of the previously damaged neurons. As shown in Figure 1 of the specification, the rats used in the human myeloid stem cell transplantation studies survived at least 20 weeks after transplantation and showed reduced apomorphine-induced rotational behavior.

Applicants provide ample guidance for identification and isolation of pluripotent myeloid stem cells (page 11, line 30 through page 14, line 2), culture of such cells (page 14, line 5-33), and transplanting these cells into *in vivo* animal models of neurological disorders, such as Parkinson's disease, stroke, and demyelinating injuries (page 15, line 1 through page 18, line

23). Furthermore, Applicants demonstrate that following implantation of human stem cells and progenitor cells of myeloid origin, the cells migrate into multiple target regions, engraft in the CNS, and differentiate into developmentally and regionally appropriate cells, such as neuronal and glial cells, which preferentially migrate to the damaged areas (page 23, line 21 through page 24, line 2). Thus, Applicants detail the methods of stem cell implantation as well as a behavioral model to demonstrate success. Based on the adequate amount of teaching and guidance provided by Applicants' specification and the well-recognized animal models, the skilled artisan could readily adapt the invention for use in mammals using the same methodology and without undue experimentation.

The Office Action also refers to the Björklund reference (PNAS Björklund *et al.* Vol. 99 No. 4, 2002) to support the position that:

> While the results of this study are intriguing, they also reveal the need for additional research. The transplanted cells did not survive in 6 of the 25 rats treated, and 5 of the animals developed tumors near the site of the transplants within the first 9 weeks. These complications illustrate the importance of learning how to control the differentiation and proliferation of stem cells before planning similar therapies in humans. See e.g. page 2349, first sentence, second column.

Applicants respectfully disagree with the basis of this rejection. Although 5 of the 25 rats implanted with ES cells developed tumors, and 6 of the 25 rats did not result in a successful cell implantation, the remaining 14 rats not only survived 14-16 weeks, but were successfully treated following stem cell implantation. Results of studies on these 14 treated rats illustrate that "DA neurons can develop *in situ* from implanted naive ES cells....[and, can]...reinnervate the host brain, reduce motor asymmetry, and restore physiological functional MRI response to DA-releasing agents." (See page 2348, column 1, paragraph 2). Furthermore, the study showed that ES cells can differentiate into different types of neurons, such as DA neurons and 5HT neurons (See page 2348, column 1, paragraph 5), dependent upon the location of the injected cells. Thus, contrary to the Examiner's position, Applicants contend that the experiments described in Björklund *et al.* 2002 were successful in demonstrating "efficient ES cell transplantation, expansion, and differentiation into functional DA neurons." (See page 2349, column 2,

paragraph 2). In his commentary on the Björklund *et al.* 2002 article, Freed supports Applicants' position that the results of the experiments of Björklund *et al.* "illustrate the principle that relatively undifferentiated cells can develop into neurons appropriate for a specific brain region..." (Freed, C. R. *PNAS* 99(4):1755-1757 (2002); See page 1755, column 2). For all the forgoing reasons, the Examiner is respectfully requested to withdraw the rejection.

CONCLUSION

In summary, the above-identified patent application has been amended and reconsideration is respectfully requested for all the reasons set forth above. In the event that the amendments and remarks are not deemed to overcome the grounds for rejection, the Examiner is kindly requested to telephone the undersigned representative to discuss any remaining issues.

Respectfully submitted,

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Date: August 20, 2002

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